

CLAIMS

1. A method for the purification of a virus from a host cell, said method comprising in the given order the steps of:
  - 5 a) culturing host cells that are infected with a virus,
  - b) adding nuclease to the cell culture, and
  - c) lysing said host cells to provide a lysate comprising the virus.
- 10 2. A method according to claim 1, said method further comprising:
  - d) clarification of the lysate.
- 15 3. A method according to claim 1 or claim 2, said method further comprising:
  - e) further purifying the virus with at least one chromatography step.
- 20 4. A method according to any one of claims 1-3, wherein said virus is a recombinant adenovirus.
5. A method according to any one of claims 1-4, wherein the nuclease of step b) is Benzonase.
- 25 6. A method according to any one of claims 1-5, wherein step c) of lysing the host cells is performed with a detergent.
- 30 7. A method according to claim 6, wherein the detergent is Triton-X100.

8. A method according to any one of claims 2-7, wherein step d) comprises depth filtration and membrane filtration.
9. A method according to claim 8, wherein the membrane  
5 filtration is performed using a combination of 0.8µm and 0.45 µm filters.
10. A method according to any one of claims 3-9, wherein  
10 prior to step e) the clarified lysate is subjected to ultrafiltration and/or diafiltration.
11. A method according to claim 10, wherein the clarified  
lysate that is subjected to diafiltration is exchanged  
15 against a solution comprising 0.8-2.0 M NaCl, preferably about 1 M NaCl, or another salt providing an equivalent ionic strength.
12. A method according to any one of claims 4-11, wherein  
20 step e) comprises anion exchange chromatography.
13. A method according to claim 12, wherein said anion  
exchange chromatography is performed using a charged  
filter comprising anion exchange groups.
- 25 14. A method according to any one of claims 4-13, wherein  
step e) comprises size exclusion chromatography.
15. A method according to any one of claims 4-14, wherein  
30 step e) comprises:  
e,i) anion exchange chromatography, and  
e,ii) size exclusion chromatography.

16. A method according to claim 15, wherein the mixture  
containing the recombinant adenovirus is buffer exchanged  
with a solution comprising at least 2 M NaCl, or another  
salt providing an equivalent ionic strength, between said  
5 steps of anion exchange chromatography and size exclusion  
chromatography.
17. A process according to any one of claims 4-16, wherein  
the buffers used in steps d) and subsequent steps are  
10 free of detergent, magnesiumchloride and sucrose.
18. A method for the purification of a virus that is capable  
of lysing host cells, said method comprising the steps  
of:  
15 a) culturing host cells comprising said virus capable of  
lysing host cells,  
b) harvesting virus following their release into culture  
fluid without addition of an external lysis factor,  
characterized in that a nuclease is added to the culture  
20 before 95% of the host cells has been lysed.
19. A method for the production of a virus comprising a  
nucleic acid sequence coding for a nucleoprotein of a  
hemorrhagic fever virus, comprising the steps of:  
25 a) culturing host cells that have been infected with said  
virus,  
b) subjecting said culture of host cells comprising said  
virus to lysis of the host cells to provide a lysate  
comprising said virus,  
30 c) subjecting the virus to anion exchange chromatography,  
characterized in that after anion exchange chromatography  
the virus containing mixture is buffer exchanged with a

solution comprising at least 1 M NaCl, or another salt providing an equivalent ionic strength and/or with a solution comprising at least 1% of a detergent.

- 5      20. A method according to claim 19, wherein the virus containing mixture is buffer exchanged at least once with a solution comprising at least 1 M NaCl, or another salt providing an equivalent ionic strength.
- 10      21. A method according to claim 19 or claim 20, wherein said virus is a recombinant adenovirus.
22. A method according to any one of claims 19-21, wherein said heamorrhagic fever virus is Ebolavirus.
- 15      23. A method according to any one of claims 20-22, wherein said solution comprises at least 1.5 M NaCl, or another salt providing an equivalent ionic strength.
- 20      24. A method according to claim 23, wherein said solution comprises at least 2 M NaCl, or another salt providing an equivalent ionic strength.
- 25      25. A method according to claim 24, wherein said solution comprises at least 3 M NaCl, or another salt providing an equivalent ionic strength.
- 30      26. A method according to claim 25, wherein said solution comprises about 5 M NaCl, or another salt providing an equivalent ionic strength.

27. A method according to any one of claims 19-26, further comprising filtering the virus containing mixture that is buffer exchanged through a hydrophilic filter with a pore size of 1.2  $\mu\text{m}$  or less.
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28. A method according to claim 27, wherein said pore size is about 0.45  $\mu\text{m}$  or about 0.22  $\mu\text{m}$ .
29. A method according to any one of claims 19-28, further comprising subjecting the virus containing mixture that is buffer exchanged to size exclusion chromatography.
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30. A method for removing free adenovirus proteins from a recombinant adenovirus preparation, comprising the step of: subjecting a recombinant adenovirus preparation comprising free adenovirus proteins to a charged filter that contains anion exchange groups.
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31. A method according to claim 30, wherein said recombinant adenovirus preparation comprises a subgroup B recombinant adenovirus.
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32. A method according to claim 30 or claim 31, wherein said recombinant adenovirus is an Ad35 recombinant adenovirus.
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